

The University of Chicago

THE RELATIVE CUMULATIVE ACTION
OF CERTAIN SQUILL GLUCOSIDES,
OUABAIN, AND DIGITALIS

A DISSERTATION SUBMITTED TO THE FACULTY
OF THE DIVISION OF THE BIOLOGICAL
SCIENCES IN CANDIDACY FOR THE DEGREE
OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF PHYSIOLOGICAL CHEMISTRY
AND PHARMACOLOGY

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By
EDWARD WILLIAM WALLACE

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CUMULATIVE POISONING BY SQUILL DERIVATIVES AND BY OUABAIN

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Cumulative poisoning by digitalis bodies has been studied by several methods among which the best known and most used is that of Hatcher (1). The usual technique of this author was to inject a digitalis body intravenously, then to allow an interval of one day to several weeks elapse, and finally to gauge the degree of cumulation by determining the lethal dose of ouabain by the intravenous injection technique commonly used since his work (2). The greater the degree of cumulative poisoning caused by a given glucoside or mixture of glucosides, the less was the dose of ouabain finally required. In the investigation here reported, however, the method employed resembles that of Fraenkel (3). Dogs have been given an intravenous constant dose of a given glucoside, the effects of which were observed by clinical symptoms and by electrocardiograms. The degree of cumulative poisoning produced was considered to be inversely proportional to the mean survival period.

The following digitalis bodies were used in the study of cumulation: scillonin and scillicin,¹ scillaren A and B,² ouabain (U. S. P.),³ and one tincture of digitalis made in this laboratory. Scillicin and scillaren B are amorphous bodies which, like the crystalline glucoside ouabain, are soluble in water. Scillonin and scillaren A are crystalline bodies which must be dissolved in ethyl alcohol before dilution by an aqueous medium. Scillonin is a dextrorota-

¹ Furnished by the Grisard Laboratories.

² Furnished by the Sandoz Company.

³ Furnished by the Bureau of Food and Drug Administration, Department of Agriculture.

tory glucoside (in 95 per cent ethyl alcohol) and melts at 220°C.; its empirical formula is considered by the manufacturer to be $C_{40}H_{52}O_8$. Scillaren A is levorotatory (in 75 per cent ethyl alcohol); the empirical formula given to Scillaren A by the manufacturer is $C_{36}H_{52}O_{13}$.

EXPERIMENTAL METHODS

The method of assaying the glucosides for their cat and dog lethal doses is a modification of the one used originally by Hatcher and Brody (2). The animals were lightly anesthetized with ether, a cannula was inserted into the trachea, and artificial respiration was applied. This last procedure was routinely followed to prevent the possible occurrence of death from respiratory failure during the later stages of administration of the drug. The blood pressure was recorded from the carotid artery by means of a mercury manometer. Suitable dilutions of the drug in normal saline were then allowed to flow from a burette into the femoral vein at a constant rate so that the animal died in thirty to forty-five minutes. The final fall of the blood pressure to zero was taken as the endpoint. Care was taken in each case not to use animals which were pregnant or animals which had been fed twenty-four hours previous to the experiment.

For the frog assay, a special constant temperature bath designed in our laboratories by Foster and van Dyke (4) was used in conjunction with a modified Smith-McClosky (5) intravenous technique. The constant temperature bath consisted of a series of small compartments built in tiers through which water was made to flow. A thermostatic unit kept this water constantly at 20°C. The frogs (*Rana pipiens*) were introduced into the compartments one hour before the beginning of the assay. Each frog in a numbered compartment was then pithed, weighed, and given an injection into the musculocutaneous vein. The dose chosen was based upon weight and was estimated, after a preliminary experiment, to kill approximately 50 per cent of a group. Two hours after the injection the hearts were exposed and observed. A heart which contracted automatically, however slowly, or contracted in response to successive mechanical stimuli was recorded

as negative. A heart which was found to be in systolic contracture or one which passed into this condition following not more than three successive mechanical stimulations was recorded as positive.

Twenty frogs were used in the assay of each drug. A sufficient number of frogs was employed so that all the drugs could be assayed simultaneously. In this way any one of them could be used as a standard of comparison in calculating relative doses. From the per cent mortality, observed in 20 frogs, the 50 per cent lethal dose was calculated by reference to a relative dose curve previously determined (4). Both the absolute and relative doses were based on this figure.

The solutions used in the cumulation experiments were prepared every twenty-four to forty-eight hours. They were stored in the dark at 4°C. when not in use. A calibrated Hartmann and Braun torsion balance which had a capacity of 10 mgm. and could be read with an accuracy of ± 0.005 mgm. was used to weigh the quantity of glucoside desired. The solutions were prepared in 50 cc. volumetric flasks at room temperature. Calculated volumes (based on previous assay) of digitalis tincture were measured directly from 1 cc. Koch pipettes into small beakers and thence aspirated and washed into the syringes with normal saline. The stock bottle of digitalis tincture was sealed with paraffin in addition to being kept cool and away from light.

Only medium sized adult dogs in good health were selected for the cumulation experiments. Pregnant, fat, very young, very old, emaciated or diseased dogs were avoided. During the course of an experiment their cages were washed daily with tricresol. The ration consisted of meat, milk, dog biscuit, and some vegetable. Every effort was made to keep the animals in as good a nutritional and hygienic state as possible. Results obtained with dogs developing a respiratory infection during the experimental period, in spite of these precautions, were discarded.

The antebrachial vein of the foreleg was found to be very convenient for the intravenous injections. In 1 case in which this vein was too small the jugular vein was used in its place. In prep-

aration for an experiment a dog's forelegs and left hind leg were shaved and thoroughly washed. He was then trained to lie quietly on a small table so that smooth and regular records, with no distortion of the components as a result of body movements, could be obtained with the electrocardiograph. This could be facilitated by covering the animal and shielding him from light.

Each dog was given a daily intravenous injection of a certain percentage of a lethal dose, which was based upon his weight and the cat assay for the glucoside studied, until death occurred. No anesthetic was at any time employed. The comparative lethality of the various glucosides for the cat and dog are discussed later. The course of the chronic poisoning was followed daily by electrocardiograms, leads I and III, both before and after the administration of the drug, and by coincident clinical observations of the pulse and of gastro-intestinal and respiratory symptoms. The electrocardiograms were made some minutes before as well as thirty minutes after each daily injection; tracings made after longer periods appeared to furnish no additional information on the maximal effects produced by the single daily injection. The attempt was made to inject regularly at the same time each day, and also to take the electrocardiograms at the same time intervals relative to injection, except when there was interference by unusual asphyxial or convulsive seizures. In this way it was possible to obtain fairly comparable pictures of the progressive intoxications. At death necropsies were performed in order to rule out infection or factors other than the toxic effects of the drug injected as contributory causes of death.

TOXICITY OF GLUCOSIDES USED

A summary of data obtained from the assay of the various glucosides in the cat is shown in table 1. The lethal dose and standard deviation are expressed in milligrams per kilogram. Because of the possibility that the tincture of digitalis might lose some of its activity upon standing, redeterminations of its lethal dose were made during the course of the experiment and at its end. No appreciable deterioration occurred in that time (2G, 3G). That

at least two of the other glucosides, kept as solids in a desiccator in the dark at 4°C., did not deteriorate may be seen by the re-determinations of their toxicity (2A, 2B, 2C).

Determinations of the lethal doses, for the cat and dog, of some of the glucosides employed are compared in table 2. A different digitalis sample was used for the comparison. In all cases except that of scillaren B, the mean lethal dose for the dog is not significantly different from that for the cat; the dog appears to be more resistant than the cat to this drug. Therefore it was felt that

TABLE 1
The lethal dose of certain digitalis bodies for the cat

DRUG	EXPERIMENT	NUMBER OF ANIMALS	MEAN L.D. AND STANDARD DEVIATION	DOSES TAKEN AS LETHAL FOR CUMULATION EXPERIMENTS
			<i>mgm. per kgm.</i>	<i>mgm. per kgm.</i>
Ouabain*.....	1A	5	0.103 $\pm$ 0.015	0.100
	2A	5	0.095 $\pm$ 0.017	
Crystalline scillonin.....	1B	6	0.098 $\pm$ 0.013	0.100
	2B	2	0.121 $\pm$ 0.006	
	1C	5	0.100 $\pm$ 0.031	
	2C	3	0.094 $\pm$ 0.012	
Scillaren B.....	1D	6	0.109 $\pm$ 0.017	0.100
Scillaren A.....	1E	7	0.139 $\pm$ 0.037	0.139
Scillicin.....	1F	7	0.213 $\pm$ 0.024	0.213
Digitalis.....	1G	5	67.3 $\pm$ 10.0	67.0
	2G	3	67.0 $\pm$ 2.2	
	3G	9	81.6 $\pm$ 15.0	

* All doses are expressed in terms of U. S. P. (not anhydrous) ouabain.

with the possible exception of scillaren B, the routine use of the cat lethal dose was not objectionable as a dosage-basis for the comparative cumulative experiments. In none of the data (including electrocardiograms) of the acute experiments could be found any evidences of qualitative differences among the various glucosides used. The results of one of the frog assays of these substances are given in table 3. As was mentioned above, all these bodies were assayed simultaneously, so that any one of them may be used as a standard of comparison. Here, ouabain served as the standard in calculating the relative dose. The possible relation-

ship of cumulation to frog and mammalian lethal doses will be discussed later.

TABLE 2

The comparative lethality of certain digitalis bodies for the cat and dog

DRUG	SPECIES	NUMBER OF ANIMALS	MEAN AND STANDARD ERROR OF MEAN LETHAL DOSE	P*
Ouabain.....	Cat	7	0.100 $\pm$ 0.006	0.32
	Dog	8	0.116 $\pm$ 0.013	
Scillonin.....	Cat	8	0.103 $\pm$ 0.007	0.07
	Dog	6	0.083 $\pm$ 0.007	
Scillaren B.....	Cat	6	0.109 $\pm$ 0.007	0.01
	Dog	12	0.144 $\pm$ 0.008	
Scillaren A.....	Cat	7	0.139 $\pm$ 0.014	0.50
	Dog	4	0.124 $\pm$ 0.012	
Digitalis.....	Cat	9	79.9 $\pm$ 7.35	0.96
	Dog	10	80.1 $\pm$ 3.71	

* Probability that random sampling would yield as great a difference.

TABLE 3

The lethal dose of certain digitalis bodies for the frog

DRUG	NUMBER OF FROGS	ABSOLUTE L.D. FOR 50 PER CENT OF GROUP	RELATIVE L.D.*
		<i>mgm. per kgm.</i>	
Ouabain.....	20	0.359	1.00
Crystalline scillonin.....	20	0.782	2.18
Scillaren B.....	20	0.344	0.96
Scillaren A.....	20	0.471	1.31
Scillicin.....	20	1.150	3.20
Digitalis.....	20	149.0	415.0

* Using ouabain as a standard.

CUMULATION EXPERIMENTS

Protocols representative of the cumulation experiments are summarized in tables 4 and 5. In these particular examples, ouabain and scillonin were used.

TABLE 4
A cumulation experiment with ouabain
 (Thirty-five per cent of a lethal dose was administered daily)

DOG 52		ELECTROCARDIOGRAMS					PHYSIOLOGICAL EFFECTS						
Day	Relation to injection	Rate	Conduction time		S-T complex	Extra-systoles	Heart block	Conditioned salivation	Salivation	Hyperpnea	Retching or vomiting	Cyanosis	Asphyxial struggles
			A-V	Q.R. S.									
			sec-onds	sec-onds									
1	{ 3 minutes before 30 minutes after	100	0.06	0.04	Normal	—	—	—	++	0	0	—	—
		54	0.06	0.04									
2	{ 3 minutes before 30 minutes after	83	0.06	0.04	Normal	—	—	—	++	1*	1*	—	—
		46	0.09	0.08									
3	{ 3 minutes before 30 minutes after	70	0.08	0.06	Normal	—	—	+	++	1	1	—	—
		57	0.10	0.08									
4	{ 3 minutes before 30 minutes after	68	0.11	0.06	Normal	—	—	+	++	2	0	+	—
		220	0.10										
5	{ 3 minutes before 30 minutes after	64	0.16	0.06	Normal	—	Partial 2-1	+	++	1	0	+	—
		46	0.06	0.06									
6	{ 3 minutes before 30 minutes after	61	0.14	0.06	Low potential	—	Partial 2-1	+	++	2	0	+	—
		42	0.08	0.08									
7	{ 3 minutes before 30 minutes after	70	0.16	0.06	Low potential	—	Partial 2-1	+	++	1	0	+	—
		65	0.07										
8	{ 3 minutes before 9 minutes after 30 minutes after	46	0.06	0.06	Low potential	—	Complete A-V	—	++	2	0	+	—
		210	0.08	0.08									
9	{ 3 minutes before 30 minutes after	46	0.06	0.06	Low potential	V	Complete A-V	—	++	2	0	+	—
		210	0.08	0.08									

Necropsy: No gross pathology of internal organs.

* Number of seizures of hyperpnea or vomiting.

† Extrasystoles arising in left ventricle.

In the experiment with ouabain, the heart rate was steadily reduced each day. The P-R interval and the duration of the Q.R.S. complex during this time progressively increased until complete heart-block finally supervened. The condition of complete heart-block was especially seen shortly after the injection of the drug; usually it would be replaced by various grades of partial heart-block by the next day. Toward the end of the chronic poisoning apparently a sufficient amount of the glucoside had cumulated in the heart to keep it permanently in a condition of complete block. At this stage, also, evidences of altered ventricular physiology could be seen in the appearance of abnormal Q.R.S. complexes; these were characteristically of high potential and of bizarre and irregular contour.

After a few injections a marked salivation followed the administration of the drug. This has also been observed in the cat by Klein (6). The animals also would frequently vomit and show signs of incompetent circulation such as dyspnea, cyanosis, and asphyxial struggles. Frequently the animals exhibited a conditioned salivation simply upon being led into the room in which the injections were made.

The description of the symptoms resulting from chronic administration of ouabain is essentially the same as those which could be given for digitalis, scillaren A and B, and scillicin.

The course of the experiments with crystalline scillonin was somewhat different. Here the survival period was longer although the amount of glucoside injected per day was much greater (table 5). There was no consistent daily drop in the pulse rate. In fact the pulse rate tended to increase in the later stages of poisoning. The P-R and Q.R.S. intervals also remained practically unchanged, until near the end when they increased. Very shortly (five to ten minutes) after administering this drug, however, there was a marked vagal slowing of the pulse. Electrocardiograms taken at this time showed a very marked lengthening of P-R and Q.R.S. intervals. In some cases the P wave disappeared entirely from the record and only occasional Q.R.S. and T complexes could be seen. Coincident with these electrocardiographic changes the animal retched, became progressively more dyspneic and cyanotic

TABLE 5
A cumulation experiment with crystalline scillonin
 (Seventy per cent of a lethal dose was administered daily)

Dog 77			ELECTROCARDIOGRAMS					PHYSIOLOGICAL EFFECTS					
Day	Relation to injection	Rate	Conduction time		S-T complex	Extra-systoles	Heart block	Conditioned salivation	Salivation	Hyperpnea	Retching or vomiting	Cyanosis	Apnoeal struggles
			A-V	Q.R.S.									
1	3 minutes before	103	0.08	0.04	Normal	—	—	—	+	1	1	—	—
	9 minutes after	120			High potential +	L.V.	—	—					
	30 minutes after	120	0.10	0.04	Negative	—	—	—	+	0	0	—	—
2	3 minutes before	83	0.08	0.04	Normal	—	—	—	+			—	—
	30 minutes after	73	0.10	0.04	Normal	—	—	—					
3	3 minutes before	77	0.09	0.04	Normal	—	—	—	+	2	2	—	—
	30 minutes after	67	0.11	0.04	Normal	—	—	—	++				
4	3 minutes before	68	0.10	0.04	Normal	—	—	—	+			—	—
	30 minutes after	61	0.10	0.04	Normal	—	—	—	++	1	1	—	—
5	3 minutes before	68	0.10	0.04	Normal	—	—	—	++	1	1	—	—
	10 minutes after	33			High potential +	L.V.	Complete S-A	—	+++			+	—
	30 minutes after	115	0.10	0.04	Negative	L.V.	—	—					
7	3 minutes before	60	0.10	0.04	Normal	—	—	—	++	1	1	+	+
	12 minutes after	40			Normal (Normal)	+	—	—	+++				
	30 minutes after	45	0.12	0.04	Low potential +	—	—	—	+++				
8	3 minutes before	82	0.10	0.04	Normal	—	—	—	++	1	1	+	+
	30 minutes after	180	0.10	0.04	Negative	L.V.	—	—	+++				

TABLE 5—Continued

9	3 minutes before 11 minutes after 30 minutes after	107 45-0 80	0.10 0.10 0.04	Normal High potential + Normal	L.V. —	A-V to S-A —	++	+++	1	+	+
10	3 minutes before 13 minutes after 30 minutes after	110 40-0 91	0.10 0.04 0.04	Normal High potential + Normal	L.V. —	A-V to S-A Complete A-V	++	+++	1	+	+
11	3 minutes before 7 minutes after 30 minutes after	71 40-0 77	0.10 0.04 0.04	Normal High potential Normal	L.V. L.V.	A-V to S-A —	++	+++	1	+	+
12							++	+++	1	+	+
13	3 minutes before 30 minutes after	136 150	0.10 0.12 0.04	Normal Normal	— —	— —	++	+++	1	+	+
14	3 minutes before 12 minutes after 30 minutes after	130 40-0 210	0.10 0.05 0.05	Normal High potential Large S	L.V. L.V.	A-V to S-A Complete A-V	++	+++	1	+	+
15	3 minutes before 9 minutes after 30 minutes after	112 40-0 81	0.12 0.04 0.14 0.06	Low potential + Low potential + Diphasic	L.V. —	A-V to S-A Partial 2-1	++	+++	1	+	+
16	3 minutes before 30 minutes after	112 60	0.11 0.06 0.14 0.05	Low potential + Large S	— —	Partial 2-1	++	+++	1	+	+
17	3 minutes before 10 minutes after 30 minutes after	120 40-0 62	0.11 0.06 0.16 0.06	Large S High potential Diphasic	L.V. —	A-V to S-A Partial 2-1	++	+++	1	+	+
18	3 minutes before 10 minutes after 30 minutes after	73 40-0 107	0.11 0.06 0.16 0.06	Large S High-low potential Diphasic	L.V. —	A-V to S-A —	++	+++	1	+	+
19							++	+++	1	+	+

Necropsy: No gross pathology of internal organs.

and struggled as if in an asphyxial state. This condition passed off rather quickly and was followed by an increased heart-rate and various grades of block, from partial to complete. Ventricular extrasystoles were also present. By the next day the electrocardiographic complexes were normal and the heart had completely recovered.

In a few experiments in which atropine was given intravenously at the height of the asphyxial seizure, the animal recovered imme-

TABLE 6
The cumulation of certain digitalis bodies in the dog

EXPERIMENT NUM- BER	DRUG	ANIMALS			DAILY DOSE			MEAN SURVIVAL PERIOD AND STANDARD ERROR OF MEAN	SIGNIFICANCE OF DIFFERENCE*	
		Number	Mean weight		Absolute	In terms of L.D. for			Experi- ments com- pared	P†
			At start	At death		Cat	Dog			
			kgm.	kgm.		mgm.	per cent	per cent		
1	Ouabain	8	10.7	9.3	0.035	35	30	8.3 ±2.00	1-3 1-4	0.74 0.67
2	Scillonin	8	8.6	8.3	0.070	70	84	20.9 ±2.66	1-5 1-6	0.90 0.96
3	Scillaren B	8	13.9	12.2	0.025	25	17	9.0 ±1.05	4-6	0.50
4	Scillaren A	8	9.2	7.7	0.035	25	28	9.6 ±1.27	2-1 2-3	0.002 0.001
5	Scillicin	8	10.0	9.1	0.053	25		8.5 ±0.60	2-4 2-5	0.002 0.0004
6	Digitalis	8	8.8	7.5	16.75	25		8.4 ±1.30	2-6	0.0008

* Differences in mean survival periods alone considered.

† Probability that random sampling would give as great a difference.

diately; the heart and respiratory rates became normal. This may be considered as additional evidence of the vagal character of the cardiac slowing. Both the conditioned salivation and the salivation following injection exceeded those produced by the other glucosides. Conditioned emesis even occurred occasionally.

A group of 8 dogs was used for each glucoside. The mean survival period of each group was taken as the chief indication of the cumulative power of each drug. These data are summarized in

table 6. It is seen that in terms of the lethal doses for the cat, the dogs received 0.7 lethal dose of crystalline scillonin, 0.35 lethal dose of ouabain and 0.25 lethal dose of the other glucosides daily. Nevertheless, the mean period of injection required for crystalline scillonin was longer and the mean loss in weight of the dogs was less than for any other drug.

If one considers for the moment only the mean survival periods, apart from the doses injected, and compares them statistically according to the method of Fisher (7) it is seen that there are no significant differences except in the case of scillonin (table 6). Other work in this laboratory indicates that in comparative assay of digitalis bodies in groups of about 10 cats each, significance cannot be attached to differences in mean results, unless the probability of the occurrence of such a difference in random sampling is 0.02 or less. Perhaps such a test of significance would be applicable to the cumulation experiments. Only in the case of scillonin as compared with the other glucosides is there a significant lengthening of the survival period, if one leaves the relative lethal dose out of consideration. Scillonin, however, was administered in twice the dosage of ouabain and in nearly three times (2.8) the dosage of the other glucosides. This fact makes the difference between scillonin and the other glucosides even more significant.

Ouabain seems definitely less cumulative than the glucosides other than scillonin since it was necessary to ingest 40 per cent (0.10 lethal dose) more of this drug to shorten the survival period to one comparable with those following the administration of 0.25 lethal dose of scillaren A, scillicin and digitalis daily. If ouabain is administered as 0.25 of the lethal dose per day, the animals live for long periods; two such animals survived three months when the experiment was discontinued. These findings, therefore, confirm the results of other workers (1, 8).

DISCUSSION

The experiments described above are believed to furnish semi-quantitative data on the comparative cumulation of some of the glucosides which can be isolated from squill. Only one sample of

digitalis tincture was used, not as being representative of all digitalis, but simply for comparison with ouabain and the squill glucosides. The drugs were administered once daily by an intravenous method so that variable absorption did not affect the results. The attempts made to prevent extraneous factors, such as respiratory infection, from contributing to death have been described above. The course of the progressive poisoning in each case, was observed from the symptoms and from electrocardiographic records. The toxicity of substances such as tincture of digitalis, which might undergo deterioration, was redetermined occasionally.

TABLE 7

A comparison of the cumulative effects in the dog with the relative lethality for the frog and cat and for the frog and dog

DRUG	FROG CAT RATIO		FROG DOG RATIO IN TERMS OF ABSOLUTE DOSES	CUMULATION	
	Relative*	In terms of absolute doses		Per cent of cat L.D. injected daily	Survival
					<i>days</i>
Ouabain.....	1.00	3.59	3.09	35	8.3 $\pm$ 2.0
Scillonin.....	2.18	7.82	9.42	70	20.9 $\pm$ 2.7
Scillaren B.....	0.88	3.44	2.39	25	9.0 $\pm$ 1.1
Scillaren A.....	0.94	3.39	3.80	25	9.6 $\pm$ 1.3
Scillicin.....	1.50	5.40		25	8.5 $\pm$ 0.6
Digitalis.....	0.62	2.22		25	8.4 $\pm$ 1.3

* Using ouabain as a standard.

If one considers the mean survival period as being inversely proportional to the degree to which a given glucoside (or group of glucosides) cumulates, it appears that in the group of drugs studied, scillaren A and B, scillicin, and tincture of digitalis cumulate most readily and are perhaps 40 per cent more cumulative than ouabain. The experiments therefore do not confirm the statements of Straub (9) and of Okushima (10) that scillaren is less cumulative than other digitalis bodies. Scillonin is remarkably non-cumulative, being clearly less than half as cumulative as ouabain and less than one-third as cumulative as digitalis, scillaren A and B, and scillicin. The acute effects resulting from the intravenous injection of scillonin, however, are perhaps more

striking than in the cases of the other glucosides and are particularly characterized by pronounced vagal effects.

The data on the cumulative as well as acute toxic effects of the glucosides used are summarized in table 7. Ouabain was used as a standard of comparison in computing the relative toxicity of the drugs in the cat and frog. Particularly interesting is the low relative toxicity of scillonin in the frog as compared with the cat. This may be related to the fact that scillonin causes the least marked cumulative poisoning. On the other hand, scillicin appears to be less toxic in the frog than in the mammal; yet it is as adequate a cumulative poison as any of the drugs studied. The frog/cat dose-ratio of scillonin is probably significantly high; in the case of scillicin it is perhaps more doubtful that the apparently high frog/cat dose-ratio is really significantly so.

SUMMARY

1. Cumulation experiments were performed with ouabain, one tincture of digitalis, and four derivatives of squill (two crystalline, two amorphous).

2. The dog was chosen as the experimental animal. Each animal received a daily intravenous dose of a glucoside until death occurred. Electrocardiograms were made before and after each injection. The dose in each case was based upon the lethality as determined in acute experiments; these indicated that with the exception of scillaren B, there was practically no difference between the cat and the dog.

3. Scillonin, a crystalline derivative of squill, was found to be less than half as potent a cumulative poison as ouabain and probably less than one-third as potent in its cumulative effects as the other drugs used.

4. The acute toxic effects of scillonin in the mammal (continuous intravenous infusion) were as great as those of ouabain (lethal dose about 0.1 mgm. per kilogram). In the frog it was relatively the least toxic of the drugs used.

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